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METHODS FOR IDENTIFICATION OF *ANASTREPHA* LARVAE (DIPTERA: TEPHRITIDAE), AND KEY TO 13 SPECIES

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Abstract.—Detailed methods are provided for observing useful characters to distinguish among species of *Anastrepha* fruit flies in their immature stages. Additionally, a key is provided to third instar larvae of 13 species: *A. bistrigata* Bezzi, *distincta* Greene, *fraterculus* (Wiedemann), *grandis* (Macquart), *interrupta* Stone, *leptozona* Hendel, *limae* Stone, *ludens* (Loew), *obliqua* (Macquart), *pallens* Coquillett, *serpentina* (Wiedemann), *striata* Schiner and *suspensa* (Loew). There is considerable overlap in many character states among species. Discriminant analysis is necessary to distinguish among species in some couplets.

Key Words: *Anastrepha*, fruit flies, larvae, discriminant analysis

Anastrepha is a New World genus of fruit flies (Diptera: Tephritidae) comprising about 180 valid species (Norrbom and Kim 1988). Several species are major fruit pests in the American tropics and subtropics. Descriptions are available for immature stages of only 15 species, and several of these are very incomplete. The paucity of taxonomic information makes it extremely difficult to identify the larvae of *Anastrepha* (as well as those of most other fruit flies). This is especially problematical because fruits infested with larvae usually are encountered in the absence of associated adults, as is the case of most interceptions of Tephritidae at U.S. ports of entry (APHIS 1987).

Published keys or descriptive works which specifically attempt to discriminate among larvae of two or more *Anastrepha* species include Greene (1929), Phillips (1946), Berg (1979), Heppner (1984), Steck and Wharton (1988), Steck and Malavasi (1988) and Carroll and Wharton (1989). Twelve species are included in these works, but not all of them simultaneously. Berg's key (1979) is the most inclusive and treats those six species considered to be the most serious pests (*fraterculus* (Wiedemann), *ludens* (Loew), *obliqua* (Macquart), *serpentina* (Wiedemann), *striata* Schiner, and *suspensa* (Loew). Unfortunately, some of the characters used in Berg's key are very difficult to interpret. Also, the natural variability within species is such that the key often leads to an incorrect identification.

In this paper, we incorporate additional characters not previously utilized. Since the natural variability of larval characters is so poorly documented, especially among geographical regions, we are careful to note the source and sample sizes of all the material utilized to generate the key. We have ad-

dressed the problem of variation by examining specimens from more than one locality to the extent that material was available.

The economically most important species are all included, as well as several other available species whose identities were certain. Thus, the usefulness of various larval characters could be assessed across a broad taxonomic range within this large genus. The 13 species treated here represent six different species groups within the genus (Norrbom and Kim 1988). Among species classified in the same group, the amount of overlap in character states is expectedly high. For some couplets it is necessary to employ discriminant analysis to determine the species to which a particular specimen belongs.

Materials and Methods

Specimens from which the key was developed are listed below. Accurate association of larvae with their identifiable adult forms was a critical objective of this study. Identity of larvae cannot be presumed if they are taken from naturally infested fruits, since even an individual fruit may be multiply infested by more than one species. Most museum specimens are not explicitly associated with reared adults from the same collection; thus, their identity must be considered cautiously. Many larvae used in this study were bred in the laboratory from known adults. Others were taken from naturally infested fruits from which numerous adult specimens of exclusively one species were reared. Exceptions are noted below. Specimen collectors' names are given in parentheses after the collection date. Local names for host fruits are given in parentheses after their scientific names. Asterisks denote specimens used to generate linear discriminant functions. Voucher specimens of all larvae and associated adults are housed in the collections of the U.S. National Museum of Natural History, Smithsonian Institution (USNM) and/or the Department of Entomology, Texas A&M University as TAMU voucher numbers 213, 214, 219, 220, 222, 223, 225, 226 and 227.

A. bistrigata Bezzi—BRAZIL: São Paulo, Universidade de São Paulo, XI-1986 (Steck, Malavasi); 23* + 7 specimens from a laboratory culture on *Psidium guajava* L. (guava) initiated with adults reared from guava, Campinas, S.P. (See Steck and Malavasi 1988.)

A. distincta Greene—VENEZUELA: Merida, Merida, V-1988 (Steck, Norrbom); 5 specimens from *Inga* sp. BRAZIL: Bahia, Cruz das Almas, VI-1988 (Steck, Conceição); 5 specimens from *Inga* sp. MEXICO: Chiapas, Tapachula vicinity, Obregon, III-1986 (Carroll); 10 specimens from *Inga* (larval identity presumed from host relationship). HONDURAS: E.A.P., 30 km s.e. of Tegulcigalpa, V-1985 (Sequiera); 10 specimens from *Inga* (larval identity presumed from host relationship).

A. fraterculus (Wiedemann)—BRAZIL: Saõ Paulo, Itaquera, XI-1986 (Steck, Malavasi); 25* specimens from *Eugenia brasiliensis* Lam. (grumichama); larval identity based on numerous previous rearings from the same trees and from which only *fraterculus* adults emerged (A. Malavasi, personal communication). BRAZIL: São Paulo, Universidade de São Paulo, XI-1986 (Steck, Malavasi); 5 specimens from culture on artificial medium initiated with adults from São Paulo state. BRAZIL: Bahia, Santo Amaro, VI-1988 (Steck, Conceição); 5 specimens from guava. MEXICO: Chiapas, Tapachula, Metapa, III-1986 (Carroll); 15* specimens from culture on guava initiated with adults from Tapachula area. COSTA RICA: Puntarenas, Dominical, IV-1986 (Steck, Valerio); 10* specimens from *Terminalia catappa* L. (almendron). VENEZUELA: Merida, Merida area, V–VI-1988 (Steck, Norrbom); 3 specimens from *Rubus glaucus* Benth. (mora), 3 specimens from *Syzygium jambos* L. Alston (pomarrosa) and 3 specimens from *Coffea arabica* L. (café). VENEZUELA: Dto. Federal, Las Caracas,

V-1988 (Rosales); 3 specimens from *T. catappa.*

A. grandis (Macquart)—BRAZIL: São Paulo, Universidade de São Paulo, XI-1986 (Steck, Malavasi); 18 specimens from culture on *Cucurbita maxima* Duch. and 5 specimens from culture on *Cucumis melo* L. (melon) initiated with adults reared from *C. maxima* at São Roque, S.P. ARGENTINA: 11 specimens from USNM, preserved in alcohol and bearing the following label: "*Anastrepha grandis* (Macq.) Pumpkin Argentina. 1-4-37 Houston Tex.-2003 Lot. 37-521." (See Steck and Wharton 1988.)

A. interrupta Stone—USNM: 18 specimens preserved in alcohol bearing the following label: U.S.A. "*Anastrepha interrupta* Homestead, Fla. 3.i.1951 *Schoepfia chrysophylloides* berries; 51-997 SPBFLA 109425." (See Steck and Wharton 1988.)

A. leptozona Hendel—MEXICO: Chiapas, Tapachula vicinity, Huehuetan. III-1986 (Carroll); 19 specimens from *Microphollis mexicana* (Gilly) (baricoco). (Larvae of *A. serpentina* occurred in low frequency in same collection, but were readily distinguishable.)

A. limae Stone—USNM: 31 specimens preserved in alcohol bearing the following label: "PANAMA: Capira 19–20.x.1935 J. Zetek 3552 reared ex *Passiflora quadrangularis.*" (See Steck and Wharton 1988.)

A. ludens (Loew)—MEXICO: Chiapas, Tapachula, Metapa, IV-1986 (Carroll); 15 specimens from culture on *Mangifera indica* L. (mango) initiated with adults reared from mango in Tapachula area. U.S.A.: Texas, Texas A&M University. IV-1984 (Carroll); 15 specimens from culture on artificial medium. (See Carroll and Wharton 1989.)

A. obliqua (Macquart)—MEXICO: Chiapas, Tapachula, Metapa, IV-1986 (Carroll); 20* specimens from culture on mango initiated with adults reared from *Spondias* sp. (jobo) in Tapachula area. COSTA RICA: Alajuela, F. Baudrit Expt. Stn., IV-1986 (Steck, Valerio); 9* specimens from mango. VENEZUELA: Merida, Hwy 7 × Pueblo Nuevo road, VI-1988 (Steck, Norrbom, Holmquist); 5 specimens from mango. BRAZIL: Bahia, Cruz das Almas area, VI-1988 (Steck, Conceição); 2 specimens from *Averrhoa carambola* L. (carambola), 2 specimens from *Spondias purpurpea* L. (caja) and 2 specimens from mango.

A. pallens Coquillet—USNM: 10 specimens preserved in alcohol bearing the following labels: "*Pseudodacus pallens* (Coq.) / *A. pallens* / laboratory collection Coma berries *Pseudodacus pallens* Coq. lot no. 35-19611 FHB / GVH #35."

A. serpentina (Wiedemann)—MEXICO: Chiapas, Tapachula, Metapa, III-1986 (Carroll); 20 specimens from culture on *Manilkara zapota* (L.) P. Royen (chico zapote) initiated with adults from *M. zapota, Pouteria sapota* (Jacq.) Moore and Stearn (mamey) and *Chrysophyllum cainito* L. (caimito) from Tapachula area. MEXICO: Veracruz, Los Tuxtlas Biol. Stn., VII-1984 (Steck); 11 specimens from *P. sapota.* VENEZUELA: Aragua, Maracay, V-1988 (Steck, Norrbom, Rosales); 5 specimens from *M. zapota* (nispero). BRAZIL: São Paulo, São Sebastião, VI-1988 (Amaral); 5 specimens from *M. zapota* (abrico). *A. striata* Schiner—MEXICO: Chiapas, Tapachula, Metapa, IV-1986 (Carroll); 20* specimens from culture on guava initiated with adults reared from guava in Tapachula area. COSTA RICA: Cartago, Tres Equis, Hwy 10 between Turrialba and Siquirres, 3-IV-1986 (Steck, Carlson, Valerio); 10* specimens from guava. VENEZUELA: Merida, Merida, V-1988, (Steck, Norrbom, Holmquist); 5 specimens from guava. VENEZUELA: Miranda, Guatopo National Park, VI-1988 (Condon); 5 specimens from guava.

A. suspensa (Loew)—U.S.A.: Florida, Homestead, TREC-IFAS, I-1985 (Baranowski); 30* specimens from culture on artificial medium initiated with adults from southern Florida.

In developing the key, complete measurements were taken on all specimens as in Steck and Wharton (1988). Those characters newly used in this key mostly concern the presence or absence of dorsal spinules on the various segments, and quantitative counts and measurements on the posterior spiracular processes. For convenience, some of the measurement procedures are repeated here as they relate specifically to the use of the key. Only features visible with a dissecting or compound microscope were examined. Terminology follows Teskey (1981).

Oral ridge (ORL) counts and determination of anal lobe shape are taken from whole specimens. Specimens are removed from alcohol and propped in an appropriate position on an alcohol-dampened wad of cotton in a small watchglass. The alcohol evaporates off the surface after a minute or two, and the oral ridges (Fig. 1) become clearly separable and countable. Use of a strong, fluorescent light is recommended; use of an incandescent light requires careful adjustment of the lighting angle. A minimum of 80× magnification is necessary for accurate counts on most specimens. Oral ridges are counted along the inner margin adjacent to the oral opening. The terminal upper and lower oral ridges are reduced in size and often difficult to see. The shape of the anal lobes is also best determined just after the alcohol dries off the surface of the whole specimen. (Their shape usually will be still apparent after slide-mounting.) In some species the lobes are almost always obviously bilobed (e.g. *ludens, serpentina*; Figs. 3, 4); or obviously entire (e.g. *suspensa, obliqua*; Fig. 6). In other species, such as *striata* and *distincta*, the lobes may be wrinkled or finely grooved, and thus indeterminate in this respect (Fig. 5). These latter are keyed both ways at the corresponding couplets.

Anterior spiracular tubules (ANS) are also counted on whole specimens. If the spiracles are not well exposed on the whole specimen, they become so after the specimen is treated in sodium hydroxide (NaOH).

Specimens are not perfectly symmetrical; numbers of oral ridges and anterior spiracular tubules frequently are unequal on left and right sides. Count data used in the key are the average of the two sides rounded upwards; e.g. a specimen with 10 oral ridges on one side and 11 on the other would be counted as 11 (Fig. 1). (Measurements entered into discriminant analysis, however, were not rounded.) If one anal lobe is bilobed and the other entire, the specimen is considered to be bilobed.

Dorsal spinules occur in broken, parallel rows. They usually are apparent at 80× magnification with good fluorescent lighting and best seen from a dorsolateral angle. Rows are counted on the dorsum, defined to be that surface bounded by a pair of imaginary lines drawn lengthwise between the anterior spiracle and posterior spiracle on each side (Fig. 2). Many specimens have rows of dorsal spinules interrupted by a broad, bare hiatus across the medial third of the dorsum. If rows are not visible on whole specimens, one should re-check slide-mounted specimens at 100× magnification on a compound microscope. For purposes of orientation, dorsal spinules on the second thoracic segment (T2) are those immediately posterior to the insertion of the anterior spiracles.

Specimens are slide-mounted for all remaining observations. The body is slit lengthwise along one side from just below an anterior spiracle to just above the anal lobes. Specimens are then left in 10% NaOH overnight at room temperature (or about 1 hr at 60°C). Afterwards, internal tissues are easily cleared away. The cephalopharyngeal skeleton (CPS) is gently separated from the cuticle and mounted laterally as normally figured (e.g. Steck and Wharton 1988). The cuticle is mounted flat in glycerol (or permanently mounted in Hoyer's medium or Euparal). It helps to cut small notches in the

cuticle around the posterior spiracles and anal lobes, and between the anterior spiracles so the entire specimen will lie flat (see Fig. 7). Thus mounted, rows of dorsal spinules can be counted readily.

Measurements on the posterior spiracular openings (PSO) and posterior spiracular processes, SP-I and SP-IV (Fig. 8) are made at 400× magnification using an ocular micrometer. Use of Nomarski optics provides a 3-dimensional perspective and facilitates counting of trunks and tips of spiracular processes. Measurements and counts are usually made on only one side, right or left, choosing whichever side is best positioned. PSO are measured to the outside edges of the heavily sclerotized rimae; values used in the key for length (LTH) and width (WTH) are the averages of the dorsal and ventral openings. Likewise, number of tips (TIP) and trunks (TRK) is the average of SP-I and SP-IV. The number of tips usually is readily countable. Determination of the number of trunks as clearly separate insertions into the cuticle is sometimes difficult due to orientation or crowding. In practice, when the insertion points are obscured, any branch seen as separate beyond about the basal 10% is counted as a trunk. The basal width (BAS) is the distance between outermost trunks at their insertion points; again, the average of SP-I and SP-IV is used.

Throughout development of the key we tried to use ratios of measurements on related structures (e.g. basal width of SP-I and SP-IV to length of PSO) as key characters to avoid biases resulting from unusually large or small specimens (related perhaps to type of host fruit utilized). The user of this key will note, however, that numerous couplets rely on absolute measurements, e.g. length of PSO, distal width of anterior spiracles, etc. For these latter characters, ratios did not prove useful in distinguishing among species, whereas the absolute measurements did.

Very little has been published on intraspecific geographical variation in larval characters. Some of the complexity of this key arises from such variation. It is possible that other populations besides those sampled here will fall outside the key ranges. In our *ludens*, for example, the range of lengths of PSO for Weslaco specimens did not overlap with the range for specimens from Tapachula. (The key does not use this particular character in arriving at an identification of *ludens*.) Larvae of *A. fraterculus* may present an especially thorny problem, since there are long-standing, unresolved questions about the occurrence of cryptic species in different geographical regions. Larvae of other species also display non-overlapping states for various characters among assorted populations. We foresee more such problems arising as other geographical regions are sampled.

The key works strictly on the basis of morphological characters. When information on host fruit and geographic origin of specimens are available, the task of identification is considerably simplified. Table 1 summarizes host and distribution information for the 13 species included in the key.

Within-species variability was extensive, and few, if any, single characters could reliably be used to diagnose species. An adequate number of specimens was examined in most cases to allow us to delimit ranges in which most key character states fell. We aimed for a key which would allow accurate identification for 95% or more of all specimens examined. Thus, we did not include numerous additional couplets to accommodate those specimens which displayed extreme character state values. In view of the difficulties, we would consider any determination based on a single specimen to be suspect. When several specimens of a collection are examined, the likelihood of a correct determination is greatly increased.

In some cases, there was so much overlap in key character states between species, that a simple bifurcating key became unmanageable. This was true for *striata/bistrigata*

Table 1. Host plants and geographical distributions of *Anastrepha* species.

Species	Host Plants	Distribution
bistrigata	*Psidium*	southern Brazil, northern Peru
distincta	*Inga*	southern Texas to South America
fraterculus	numerous	southern Texas to South America
grandis	cucurbits	South America, Panama
interrupta	*Schoepfia*	southern Florida, Bahamas
leptozona	*Chrysophyllum, Pouteria, Micropholis*	Central and South America
limae	*Passiflora*	Venezuela, Panama, Texas
ludens	numerous	southern Texas to Costa Rica
obliqua	numerous	Mexico to South America, Caribbean
pallens	*Bumelia*	Texas to Honduras
serpentina	numerous	southern Texas to South America
striata	numerous	Mexico to South America
suspensa	numerous	Greater Antilles, Bahamas, southern Florida

and *fraterculus/obliqua/suspensa*. Multivariate statistics were employed to discriminate among species at the corresponding couplets. The data were analyzed using SAS (1988). Observations of potentially useful characters were first subjected to univariate analysis to check for normality; non-normally distributed measurements were transformed as needed. Characters were then subjected to stepwise discriminant analysis (Lachenbruch 1975). The significance level specified to enter and to keep a character was 0.05. Those characters retained as useful discriminating factors in the stepwise analysis were incorporated into discriminant analysis to develop a model for actually identifying specimens. Classification results were cross-validated by a jackknifing technique. The original models were also retested using sets of new observations on additional specimens. Specimens marked with asterisks in the preceding collections list were used to develop the discriminant models.

Results of stepwise discriminant analysis are presented in Table 2, in which relevant statistics are shown for only those characters which contributed significantly to the discriminant function. The complete sets of characters used for each stepwise discriminant analysis are as follows (significant variables are indicated by asterisks): Couplet 4′—ANS*, TRK*, $\log_{10}$(BAS)*, ORL, LTH, TIP, squareroot(WTH), RTO1 (=LTH/WTH), $\log_{10}$(RTO2) (where RTO2 = TIP/TRK), and $\log_{10}$(RTO3) (where RTO3 = BAS/LTH); couplet 14′—ORL*, ANS*, LTH*, $\log_{10}$(BAS)*, $\log_{10}$(RTO2)*, TIP, TRK, RTO1, $\log_{10}$(RTO3), squareroot(WTH); couplet 14′A and couplet 14′B—same characters as 14′, but with different significant variables; couplet 15—$\log_{10}$(BAS)*, $\log_{10}$(RTO2)*, LTH, TIP, TRK, RTO1, $\log_{10}$(RTO3), squareroot(WTH). Thus, for example, at couplet 4′, ten characters (some transformed) were analysed for discriminating *striata* and *bistrigata*. Only three characters (ANS, TRK and $\log_{10}$(BAS), indicated with asterisks, contributed significantly to the discriminant function.

The discriminant models presented in Table 3 can be used to identify individual specimens which key to the corresponding couplet. The character values for a given specimen are substituted into the equation. Whether the equation yields a positive or negative value (except couplet 14; see below) indicates to which species the specimen is most likely to belong. Consider, for example, a hypothetical specimen which keys to couplet 4′ and has 13.5 anterior spiracles (13 on one side, 14 on the other; note that values used in discriminant functions are

Table 2. Stepwise discriminant analysis results.

Step	Variable* Enter	Remove	Partial R^2	F	Prob > F
Couplet 4′: *striata* vs *bistrigata*					
1	$\log_{10}$BAS	—	0.388	28.49	0.0001
2	ANS	—	0.175	9.32	0.0038
3	TRK	—	0.167	8.64	0.0053
Couplet 14′: *suspensa* vs *fraterculus* vs *obliqua*					
1	LTH	—	0.548	49.64	0.0001
2	$\log_{10}$BAS	—	0.391	25.99	0.0001
3	ORL	—	0.294	16.68	0.0001
4	$\log_{10}$RTO2	—	0.315	18.14	0.0001
5	ANS	—	0.120	5.32	0.0068
Couplet 14′A: *fraterculus* vs *obliqua*					
1	LTH	—	0.478	58.62	0.0001
2	$\log_{10}$BAS	—	0.242	20.11	0.0001
3	ANS	—	0.110	7.68	0.0074
Couplet 14′B: *suspensa* vs *obliqua*					
1	LTH	—	0.598	65.57	0.0001
2	TIP	—	0.244	13.91	0.0006
3	ORL	—	0.137	6.69	0.0133
4	ANS	—	0.141	6.74	0.0130
Couplet 15: *fraterculus* vs *suspensa*					
1	TIP	—	0.482	62.35	0.0001
2	$\log_{10}$BAS	—	0.159	12.52	0.0007
3	$\log_{10}$RTO2	—	0.113	8.28	0.0054
4	—	TIP	0.012	0.81	0.3729

* Abbreviations: ANS, number tubules on anterior spiracles; BAS, basal width of posterior spiracular processes; LTH, length posterior spiracular opening; ORL, number oral ridges; TIP, number tips on posterior spiracular processes; TRK, number trunks on posterior spiracular processes; WTH, width posterior spiracular opening; RTO1, ratio LTH to WTH; RTO2, ratio TIP to TRK; RTO3, ratio BAS to LTH. All measurements in μm.

not rounded), 18 trunks, and a PSP basal width of 46.8 μm ($\log_{10} = 1.67$). When these values are substituted into the couplet 4′ equation, the calculation yields a value of +2.79. A positive result indicates that the specimen is *striata*; and, using the cross-validation error rate from Table 4, one would conclude that the likelihood of error is 0.231. In the case of couplet 14, a simple positive or negative result is not possible since three species are involved. A discriminant function is provided for each of the three species. Character values for an unknown specimen are substituted into each of the three equations; whichever yields the highest value (C) indicates the most likely identification. Because the natural distributions of *fraterculus* and *suspensa* do not overlap, couplets 14′ and 15 might not represent likely sets of alternatives. Therefore, we also provide discriminant analyses for the pairwise comparisons of *fraterculus* and *obliqua* (couplet 14′A), which overlap throughout mainland Central and South America, and *obliqua* and *suspensa* (couplet 14′B) which overlap in the Caribbean (Table 3).

The performance of the classification rule was examined using three error rates (Table 4): (1) the apparent error rate (errors in classifying the original specimens using the classification rule calculated from measurements on those specimens); (2) the error rate from cross-validation using a jackknifing technique; and (3) the error rate in classifying a different set of test specimens. The apparent error rate underestimates the true error rate, although this bias is reduced if the sample size is large enough. The cross-validation method using the jackknife technique is almost unbiased (Lachenbruch 1975, Panel . . . 1989). The jackknife method gives an assessment of the true probability of misclassification of additional specimens taken from the original populations. The error rate from the test data set indicates the robustness of the classification rule when applied to other populations of specimens. The classification results indicated by the apparent and cross-validation errors for each couplet of Table 4 are very similar indicating that sample sizes were adequate for developing each of the discriminant models. The model for couplet 4′ performed poorly for the test specimens of *bistrigata*, probably due to the fact that the few test specimens available were in poor condition. Also, models 14′, 14′B and 15 fared relatively poorly for *suspensa* test specimens. This indicates that the data base for *sus-*

Table 3. Linear discriminant functions.

Couplet 4':

$23.5(\log_{10}BAS) - 0.75(ANS) - 0.63(TRK) - 15.00 > 0$ *striata*
< 0 *bistrigata*

Couplet 14':

fraterculus $186.07(\log_{10}BAS) + 13.01(ORL) + 39.58(\log_{10}RTO2) + 8.63(ANS) + 1.10(LTH) - 294.42 = C_f$
suspensa $151.06(\log_{10}BAS) + 17.23(ORL) - 5.62(\log_{10}RTO2) + 8.51(ANS) + 1.25(LTH) - 285.19 = C_s$
obliqua $161.23(\log_{10}BAS) + 14.17(ORL) + 27.96(\log_{10}RTO2) + 9.77(ANS) + 1.58(LTH) - 326.32 = C_o$

Couplet 14'A:

$22.70(\log_{10}BAS) - 0.45(LTH) - 0.99(ANS) + 24.00 > 0$ *fraterculus*
< 0 *obliqua*

Couplet 14'B:

$1.40(ANS) + 0.60(T1P) + 0.24(LTH) - 2.45(ORL) - 30.93 > 0$ *obliqua*
< 0 *suspensa*

Couplet 15:

$24.20(\log_{10}BAS) + 26.67(\log_{10}RTO2) - 40.52 > 0$ *fraterculus*
< 0 *suspensa*

pensa should be augmented with specimens from additional populations.

As noted previously, it has not been possible to construct a key to accommodate all the variability observed in our samples. However, the accuracy of the key is very high. The percentage of study material which keyed correctly (*before discriminant analysis) was as follows (sample sizes for each species in parentheses): **bistrigata* (30)/*striata* (40)—100%, *distincta* (30)—97%, **fraterculus* (72)/*obliqua* (40)/*suspensa* (40)—100%, *grandis* (34)—100%, *interrupta* (18)—100%, *leptozona* (19)—95%, *limae* (31)—100%, *ludens* (30)—100%, *pallens* (10)—100% and *serpentina* (41)—98%.

The generic description and diagnosis presented below are based on published descriptions by other authors (especially Kandybina 1977) and on additional unpublished observations of our own. With the exception of holarctic species of *Rhagoletis*,

Table 4. Error rates of classification by discriminant analysis.

	Species	Apparent Error	Crossvalidation Error	Test Error (sample size)
Couplet 4':	*striata*	0.192	0.231	0.125 (n = 8)
	bistrigata	0.046	0.091	0.500 (n = 4)
Couplet14':	*fraterculus*	0.026	0.026	0.222 (n = 9)
	suspensa	0.158	0.158	0.429 (n = 7)
	obliqua	0.148	0.148	0.167 (n = 6)
Couplet 14'A: Larvae originating in Central or South America				
	fraterculus	0.025	0.025	0.214 (n = 14)
	obliqua	0.143	0.143	0.182 (n = 11)
Couplet 14'B: Larvae originating in Caribbean				
	obliqua	0.074	0.074	0.000 (n = 7)
	suspensa	0.000	0.000	0.286 (n = 7)
Couplet 15:	*fraterculus*	0.136	0.136	0.111 (n = 9)
	suspensa	0.172	0.172	0.333 (n = 9)

the larvae of relatively few species of fruit-infesting Tephritidae have been adequately described.

Larvae of numerous Diptera families may be found in fruits (Keifer 1930), though only a few of these would be found in ripe, healthy fruits suitable for human consumption. Besides Tephritidae, only a few species of Lonchaeidae are likely to be encountered. These are readily distinguished from tephritids by the appearance of the posterior spiracles. In lonchaeids they comprise a pair of prominent stumps, round, black, and heavily sclerotized, projecting from the caudal segment; rimae of spiracular openings are at nearly right angles. In tephritid fruit flies, the spiracular openings comprise a pair of three elongate slits, nearly flush with the body surface; their rimae are sclerotized and golden-brown, and their long axes are roughly parallel. Among Tephritidae, other fruit-infesting genera which may be encountered in the Neotropics and subtropics include endemic *Rhagoletis* and *Toxotrypana* and introduced species of *Ceratitis* and *Dacus*. *Rhagoletis* generally are distinctive in possessing prominent, chitinized teeth or "stomal guards" adjacent to the oral opening and strongly developed intermediate and ventral tubercles on the caudal segment (see Phillips 1946, Kandybina 1977), neither of which are seen in *Anastrepha*. *Toxotrypana* is largely restricted to papaya, larvae are very large, and all caudal sensilla are greatly reduced (see Heppner 1986). *Ceratitis* and *Dacus* both may be recognized by the presence on the caudal segment of a distinct crescent-shaped ridge connecting, or just dorsad of, sensilla I1 and I2 (see Heppner 1985, Elson-Harris 1988) and conspicuous dental sclerites (see Exley 1955). The caudal ridge is lacking and dental sclerites usually are not seen in *Anastrepha* larvae. The diagnostic and key characters are for third instars only, and cannot be applied to earlier instars. Sections I to III of the key will eliminate most specimens for which the key is not intended.

Anastrepha Generic Description (third instar)

Body elongate, 4–7 times longer than wide, pointed anteriorly. Integument thin, smooth, colorless. Spinules separate, conical, in short, staggered rows (occasionally flat, blunt, basally connected in short rows); occurring in discrete fusiform areas ventrally on all abdominal segments; also dorsally in bands on T1, T2, usually T3, present or absent on abdominal segments. Antennal and maxillary sensory organs on well-developed cephalic lobes above mouthhooks. Antennal sensory organ appearing 2-segmented with basal sclerotized, cylindrical collar and apical hemispherical to conical sense organ. Maxillary sensory organ cylindrical, truncate, apically bearing peg-shaped sensoria. Oral ridges 7–30 per side, well developed. Stomal organ minute cluster of sensilla borne distally on large, simple, oblong lobe anterior to mandible. Sclerotized stomal guards absent. Cephalopharyngeal skeleton with clearly separate sclerites as follows: Mandible falciform (occasionally uncurved and blunt), single-toothed, length to height ratio (lateral view) about 1.0–1.5, heavily sclerotized; dental sclerite apparently lacking or small and inconspicuous; epipharyngeal and labial sclerites present; hypopharyngeal sclerite in dorsal view H-shaped, width at bridge about equal to length (ratio, 0.75–1.25), and length in lateral view about twice height, anterior forks heavily sclerotized; parastomal bar long and thin, usually bent medially, 0.75–1.0 times length of hypopharyngeal sclerite; anterior sclerite irregularly developed and shaped; dorsal cornua narrowly connected at dorsal bridge; ventral cornu trough-shaped, with 7 pharyngeal ridges. Anterior spiracle with distinct, cylindrical trunk; sharply flared and bilobed apically with numerous (9–37) tubules. Caudal segment more or less smooth and rounded; intermediate sensilla I1 and I2 on relatively developed tubercles; remaining sensilla (D1, D2, I3, L1, V1–V3) on weak

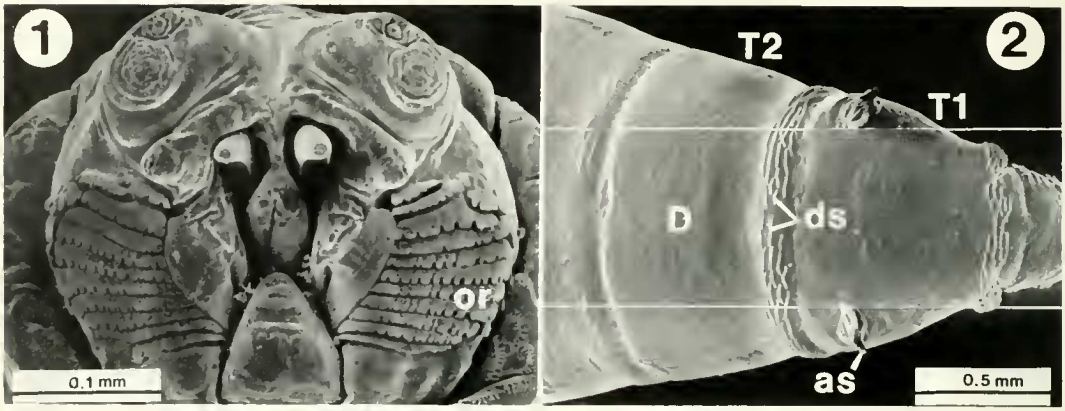


Fig. 1. Oral ridges (or), *A. suspensa.*

Fig. 2. Dorsal surface of *A. limae.* Abbreviations: as, anterior spiracle; D, dorsum bounded by imaginary lines drawn between anterior spiracles and posterior spiracles; ds, dorsal spinules in 3–4 rows on segment T2 (note hiatus in rows of spinules across mid-dorsum of segment T3); T1 and T2, first and second thoracic segments.

or undeveloped tubercles. Posterior spiracles located above horizontal midline; with three slits having well-developed rimae and trabeculae. Anal lobes entire or bifid; encircled by spinules.

Key to *Anastrepha* larvae (third instar)

I

1. Posterior spiracles prominently raised from the body surface; or most body segments with conspicuous setae or processes; or posterior spiracular openings sinuous . . . . *not* Tephritidae
1′. Posterior spiracles nearly flush with body surface; tubercles, if present, on caudal segment only; posterior spiracular slits elongate or oval II

II

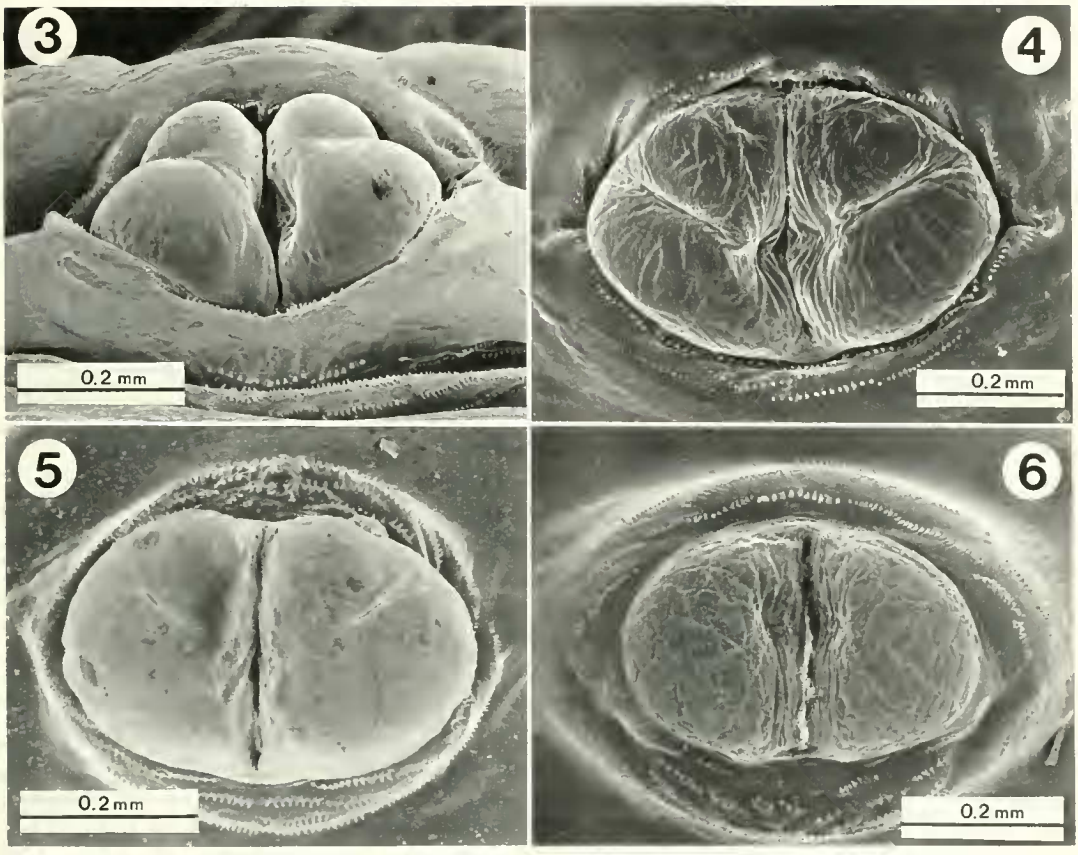
1. With prominent chitinized teeth (stomal guards) adjacent to oral opening, and strongly developed tubercles on caudal segment; or, with crescent-shaped ridge between sensilla I1 and I2 on caudal segment and conspicuous dental sclerite; or, larva taken from papaya, very large (more than 12 mm long), caudal tubercles lacking and caudal sensilla all very reduced . . . . . *not Anastrepha*
1′. Lacking stomal guards; caudal tubercles at most moderately developed; lacking crescent ridge on caudal segment; dental sclerite lacking or inconspicuous; not normally attacking papaya; at least caudal sensilla I1 and I2 conspicuous III

III

1. Anterior spiracle absent, and posterior spiracle with only 2 openings; and/or, mandible with well-developed subapical tooth, and posterior spiracular openings short (less than 55 μm) and oval; and body short and thin (less than about 6.0 mm long and 1.0 mm diameter) . . . . . *not* 3rd instar
1′. Anterior spiracle present; posterior spiracle with 3 elongate openings at least 65 μm long; mandible without subapical tooth; body length and diameter greater than about 6.0 mm by 1.0 mm IV

IV. *Anastrepha,* third instar

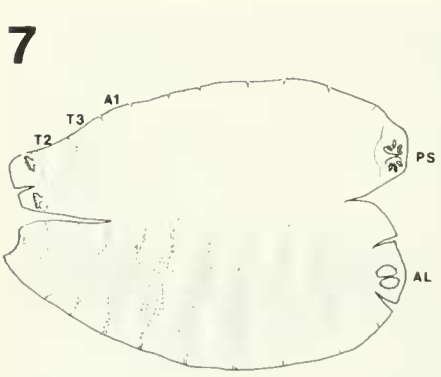
1. Dorsal spinules present on two or more abdominal segments 2
1′. Dorsal spinules present on A1, but not beyond 5
1″. Dorsal spinules absent on all abdominal segments 7
2. Dorsal spinules separate, conical; in fewer than 5–6 rows on T2 and T3 (except *limae*). Posterior spiracular processes SP-I and SP-IV with average of 6 or more trunks and bristle length ⅓ or more times length of spiracular opening 3
2′. Dorsal spinules connected basally in flat, sawtooth pattern, blunt-tipped; in 8 or more



Figs. 3–4. Bifid anal lobes, *A. serpentina.*
Fig. 5. Indeterminate, grooved anal lobes, *A. distincta.*
Fig. 6. Entire anal lobes, *A. fraterculus.*

rows on T2 and T3, at least 3 rows on A1 to A4, 1–4 rows (often with medial hiatus) on A5. SP-I and SP-IV with average of 5 or fewer trunks, and bristle length about ⅛ times length of posterior spiracular opening ..... *pallens*

3. Anterior spiracle with 12–23 tubules; distal width 0.19–0.37 mm 4
3′. Anterior spiracle with 28–37 tubules; distal width 0.43–0.61 mm ..... *grandis*
4. SP-I and SP-IV with average of 8–12 trunks and 12–21 tips; basal width 12–19 μm, 0.1–0.2 times length of spiracular opening. Dorsal spinules absent on A3 ..... *limae* (part)
4′. SP-I and SP-IV with average of 13–23 trunks and 23–49 tips; basal width 19–67 μm, 0.2–0.5 times length of spiracular opening. Dorsal spinules usually present on A3 ..... *striata* ..... *bistrigata* ..... (See Table 3, couplet 4′)
5. Dorsal spinules weakly developed on A1, in only 1 row, usually with broad medial hiatus; T2 with 2–4 rows; T3 with 1–3 rows, often with medial hiatus 6
5′. Dorsal spinules well-developed on A1, in 2 or more rows, without medial hiatus; both T2 and T3 with 5–6 rows ..... *limae* (part)
6. Anterior spiracle with 14–22 tubules. Posterior spiracular opening 94–130 μm long. SP-I and SP-IV with average of 7–13 trunks and 17–28 tips ..... *ludens* (part)*
6′. Anterior spiracle with 10–13 tubules. Posterior spiracular opening 72–84 μm long. SP-I and SP-IV with average of 4–7 trunks and 5–11 tips ..... *interrupta* (part)*
7. Oral ridges 7–11 8
7′. Oral ridges 12 or more 16
8. Anterior spiracle with 15 or more tubules 9
8′. Anterior spiracle with 9–14 tubules 14
9. Anal lobe bifid 10
9′. Anal lobe entire 11
10. Posterior spiracular opening 74–96 μm long. SP-I and SP-IV with average basal width of



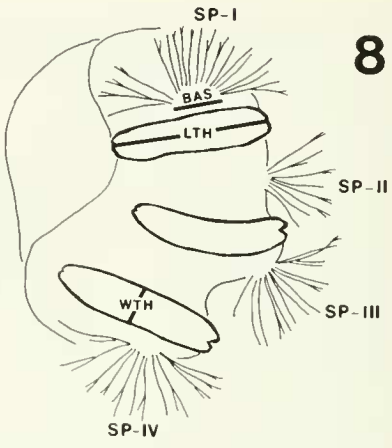


Fig. 7. Slide-mounted cuticle: AL, anal lobes; PS, posterior spiracles; T2 and T3, second and third thoracic segments; A1, first abdominal segment.

Fig. 8. Posterior spiracle (right side): SP-I to SP-IV, dorsal to ventral spiracular processes; BAS, basal width of spiracular process; LTH, spiracular opening length; WTH, spiracular opening width.

12–22 μm, 0.1–0.2 times length of spiracular opening; average of 7–11 trunks ..... *serpentina* (part)

10′. Posterior spiracular opening 103–122 μm long. SP-I and SP-IV with average basal width of 29–58 μm, 0.3–0.5 times length of spiracular opening; average of 10–17 trunks ..... *distincta* (part)

11. Anterior spiracle with 17 or more tubules .. 12

11′. Anterior spiracle with 9–16 tubules 13

12. SP-I and SP-IV with average basal width of 29–58 μm, 0.3–0.5 times length of spiracular opening ..... *distincta* (part)

12′. SP-I and SP-IV with average basal width of 14–20 μm, 0.1–0.2 times length of spiracular opening ..... *leptozona* (part)

13. SP-I and SP-IV with average of 10–17 trunks and 24–37 tips. Anterior spiracle distal width 198–273 μm ..... *obliqua* (part)

13′. SP-I and SP-IV with average of 6–10 trunks and 13–23 tips. Anterior spiracle distal width 260–335 μm ..... *leptozona* (part)

14. Anal lobe bifid 15

14′. Anal lobe entire ..... *suspensa* (part) ..... *fraterculus* (part) ..... *obliqua* (part) ..... (See Table 3, couplets 14′, 14′A, 14′B)

15. Anterior spiracle with 9–13 tubules, and SP-I and SP-IV with average of 11 or more trunks ..... *fraterculus* (part) ..... *suspensa* (part) ..... (See Table 3, couplet 15)

15′. Anterior spiracle with 13–14 tubules, and SP-I and SP-IV with average of 11 or fewer trunks ..... *serpentina* (part)

16. Anterior spiracle with 15 or more tubules .. 17

16′. Anterior spiracle with 9–14 tubules 20

17. SP-I and SP-IV with average basal width of 10–24 μm, 0.1–0.3 times length of spiracular opening 18

17′. SP-I and SP-IV with average basal width of 24–65 μm, 0.3–0.6 times length of spiracular opening ..... *distincta* (part)*

18. Anal lobe bifid 19

18′. Anal lobe entire ..... *leptozona* (part)

19. Dorsal spinules present on T3 (rows often with medial hiatus). Posterior spiracular opening 40–54 μm long ..... *ludens* (part)

19′. Dorsal spinules absent on T3. Posterior spiracular opening 31–40 μm long ..... *serpentina* (part)

20. Dorsal spinules present on T3 (rows may have medial hiatus) 21

20′. Dorsal spinules absent on T3 .. *serpentina* (part)

21. SP-I and SP-IV with average of 15 or more tips and 7 or more trunks. Up to 16 oral ridges 22

21′. SP-I and SP-IV with average of 5–11 tips and 7 or fewer trunks. Up to 20 oral ridges ..... *interrupta* (part)*

22. Anal lobe bifid. Anterior spiracle distal width 260–347 μm. Up to 16 oral ridges ..... *ludens* (part)*

22′. Anal lobe entire. Anterior spiracle distal width 161–248 μm. Up to 12 oral ridges ..... *suspensa* (part)*

* indicates that 10% or fewer of the individuals of a given species key to the corresponding couplet.

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